THE MORPHOLOGY AND LIFE-HISTORY OF THE DIGENETIC TREMATODE, MICROPHALLUS SIMILIS (JÄGERSKIÖLD, 1900) BAER, 1943 [1]

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Although significant observations had been reported earlier, the first complete life-histories of microphallid trematodes were worked out by Cable and Hunninen (1940) and Rankin (1940b). M'Intosh (1865) had described and figured larval worms from the tissues of the green crab, *Carcinides maenas* (Linnaeus), taken at St. Andrews, Scotland, but the life-cycles of trematodes were quite unknown at the time and M'Intosh described the structures as eggs, each of which contained a tiny worm that he surmised became a sexually mature distome in "such fishes as the Cotti, Gadi, and others," which feed on the crustaceans. He reported a specimen of *Cottus bulbalis,* about a foot long, with (p. 204) "two entire specimens of *Carcinus maenas,* each upwards of two inches across the carapace, in its stomach, besides the partially digested debris of others." His descriptions and figures identify the worms as members of the genus *Microphallus* and with considerable certainty as *M. similis* (Jägerskiöld, 1900). Although measurements were not given, his Figure 5 of an excysted specimen shows the suckers to be of approximately equal size and the "small globule" (seminal vesicle) anterior to the acetabulum, together with the size of the gonads, evidence full development of the metacercaria.

Levinsen (1881) described adult trematodes from the eider duck, *Somateria molissima,* taken at Egedesminde, Greenland, as *Distoma pygmaeum* and Brandes (1889) described similar worms from *Tringa alpinus* as *Distoma claviforme.* Jägerskiöld (1900) described specimens from Swedish gulls, *Larus argentatus* and *L. fuscus,* as *Distoma pygmaeum* var. *simile.* Other species have since been described from different hosts in other parts of the world. Ward (1894) described worms from American lake-fish as *Distoma opacum* and noted their similarity to *D. pygmaeum.* The parasites occurred in *Amia calva, Ictalurus punctatus* and *Perca flavescens,* and encysted larval stages were found in the crayfish, *Cambarus propinquus.* With the dismemberment of the old genus *Distoma,* Stossich (1899) erected the genus *Levinsenia* to include *Distoma brachysomum* Creplin, 1837, *D. macrophallos* von Linstow, 1875, *D. pygmaeum* Levinsen, 1881, and *D. opacum* Ward, 1894. Lühe (1899), but not Looss (1899), as has been claimed (cf. Looss, 1902: p. 704) designated *L. brachysoma,* and Jägerskiöld (1900) proposed *L. pygmaeum,* as type of the genus *Levinsenia.* Noting differences between *D. brachysomum* and *D. opacum,* Ward (1901) named the latter species type of a

[1] The experimental work was done at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the period May 1 to October 31, 1956.

new genus, *Microphallus*. In this paper he reported that the name *Levinsenia* Stossich is a homonym of *Levinsenia* Mesnil, 1897, a polychaete annelid, and that Stiles and Hassall were to propose the name *Levinseniella* to replace it. Although the paper by Stiles and Hassall did not appear until 1902, the name *Levinseniella* Stiles and Hassall in Ward, 1901 was validated with *L. brachysoma* as type. Jägerskiöld (1901), although aware of the announcement by Ward, proposed the name *Spelotrema* for the invalid name *Levinsenia,* with *S. pygmaeum* as type. Cable and Hunninen (1940), commenting on this action, wrote (p. 153), "If the law of priority should be applied to this case, *Spelotrema* Jägerskiöld, 1901 should be suppressed as a synonym of *Levinseniella* Stiles and Hassall in Ward, 1901, since Jägerskiöld stated subsequently (1904) '*Spelotrema* (= *Levinseniella*)' and therefore certainly regarded them as synonymous. His later (1907) conception of two distinct genera is valid, however, and must be accepted although he should not have retained for them names which he had regarded previously as synonyms. To suppress *Spelotrema* as a synonym of *Levinseniella,* and propose a new generic name for the species at present allocated to the genus *Spelotrema,* would probably increase rather than diminish the present confusion. For this reason, the writers are inclined to let the matter stand." As noted, *L. brachysoma* and *S. pygmaeum* are not congeneric and the characteristic features of the two genera were presented clearly by Rankin in two papers (1939, 1940a). The problem of nomenclature, stated by Cable and Hunninen, was resolved when Baer (1943) suppressed *Spelotrema* as a synonym of *Microphallus* and transferred all species from the former genus to the latter one. Baer described *Microphallus gracilis* from *Neomys fodiens* and formulated a key to thirteen members of the genus. Strandine (1943) and Rausch (1946a, 1946b, 1947) discussed morphological features and host-specificity of species in the genus *Microphallus.* Rausch and Locker (1951) described *Microphallus enhydrae* n. sp., from the sea-otter, *Enhydra lutris,* and recognized fourteen species in the genus. Stunkard (1951, 1953) described metacercariae from the horseshoe crab, *Limulus polyphemus,* and their development to sexual maturity in white mice, golden hamsters, and the herring gull, *Larus argentatus.* Although the worms agreed closely with the description of *M. claviformis* (Brandes, 1899), bionomic features seemed to preclude their allocation to that species and they were described as members of a new species, *Microphallus limuli.* Cable and Kuns (1951) erected a new genus, *Carneophallus,* and discussed the evolution and interrelationships of genera in the family Microphallidae.

Lebour (1905) reported grape-like masses of "sporocysts" in the liver of *Littorina rudis* (= *L. saxatilis*), filled with tail-less cercariae, doubled up in a curious manner. When extended, they measured 0.25 mm. in length and the figure shows them to be microphallid metacercariae. Miss Lebour regarded these larvae as identical with the encysted worms found by M'Intosh (1865) in the green crab, *C. maenas.* Brandes (1889) had suggested that the larva in the green crab is the encysted stage of *Distoma claviforme,* described by him from *Pelidna* (*Tringa*) *alpina* and *Aegialitis hiaticula.* Nicoll (1906) described specimens from the ceca and intestine of *Larus argentatus* taken at St. Andrews, Scotland, which he identified as *Levinsenia similis,* the species that Jägerskiöld (1900) had described as a variety of *Dist. pygmaeum* and (1907) raised to specific rank as type of *Spelotrema.* Nicoll noted that his specimens were somewhat larger than those of

Jägerskiöld and that worms from the ceca were larger than those from the intestine. He declared that the metacercariae from *C. maenas* are as likely to prove larvae of *S. similis* as of *S. claviforme*. The following year, Nicoll (1907) described the worms from *L. argentatus* at St. Andrews as a new species, *Spelotrema excellens,* distinct from *S. similis*. Other specimens from *Pelidna alpina, Totanus calidris* and *Aegialitis hiaticula* were described as a new species, *Spelotrema feriatum*. He gave a redescription of *S. claviforme* and in a footnote stated that the larvae from *C. maenas* are larger than the adults of *S. claviforme* and must belong to another species, perhaps *S. excellens*. The smaller metacercariae described by Lebour (1905) from the liver of *Littorina rudis* were suggested as the encysted stage of *S. claviforme*. Lebour (1908) designated the metacercaria from *C. maenas* as *Cercaria carcini* and recognized the difficulty of relating the larval forms of *Spelotrema* with any known adults. Nicoll and Small (1909) examined crabs at Millport on the Scottish west coast during August, 1908. They found three of four *C. maenas* and one of five *Cancer pagurus* infected with encysted metacercariae which they identified as a species of *Spelotrema,* most probably *S. excellens* Nicoll. At St. Andrews every green crab was infected, often with every tissue and organ riddled with cysts which occurred sometimes singly and sometimes in clusters in the liver, gonads, and along the blood vessels, nerves and intestine. They noted, as had Lebour (1908), that the cysts varied in size, shape and thickness of wall. They measured cysts of three size groups; the small ones were oval with thin walls and the largest ones were spherical with thick walls composed of inner concentric and outer radial layers. Referring to the gradual increase in size of the cysts, the authors stated (p. 239), "From these figures there seems no reason to suppose that these groups are other than stages in the growth of the same cyst, and such being the case it is evident that the cercariae increase considerably in size during their sojourn in the crab." They measured a cyst from the original material of Prof. M'Intosh which was 0.29 mm. in diameter. They suggested a possible error in the measurement given in his (1865) report, mentioned also by Guyénot *et al.* (1925).

Lebour (1912) proposed that larval trematodes should be classified on bionomic grounds and arranged the British marine cercariae in two categories, dependent on whether they were produced in sporocysts or in rediae. Among those which develop in sporocysts, she gave a more complete account of *Cercaria ubiquita,* a larva which she previously (1907) had described from *Paludestrina stagnalis* at Fenham Flats and Loch Ryan on the west coast of Scotland. She reported that this species occurred also in *Littorina saxatilis* (syn. *L. rudis*), and at Millport the usual host was *Littorina obtusata*. The cercariae resembled *C. cellulosa* and *C. pusilla* of Looss (1896) and Lebour reported that they entered *C. maenas* and *Cancer pagurus* where they developed into "Spelotrema-like cercariae." The encysted larvae occupied (p. 432) "almost every tissue of the crab, liver, muscles, gonad and outside the blood vessels. Having settled down it grows considerably and the cyst with it, but the latter however is still very thin-walled. The stylet is lost when the cyst measures about 0.30 mm. across. The ventral sucker and alimentary canal appear and the body spines begin to form. The worm stops growing when the cyst is about 0.35 mm. across and then the cyst wall becomes very thick, 0.02 mm. thick, and the real resting stage begins. The cercaria is now of the ordinary *Spelotrema* form. The usual size of the thick-walled cyst is

0.4–0.48 mm. across." After describing the excysted metacercaria, Lebour stated (p. 433), "From what has been said there can be little doubt that *Cercaria ubiquita* is the young form of *S. excellens* the first host thus being *Paludestrina stagnalis, Littorina obtusata* and *L. rudis.* The intermediate host *Carcinus maenas* and *Cancer pagurus* and the final host probably the herring gull, *Larus argentatus.*" In this paper Lebour also gave figures of *Cercaria carcini* Lebour, 1908 and *Cercaria minor* sp. inq., both from *C. maenas.* These species were encysted and therefore metacercariae; furthermore, since the cyst walls were thin and the worms somewhat smaller, it is probable, as suggested by Nicoll and Small (1909), that these larvae are identical with the ones identified by Lebour as *Spelotrema excellens.*

Guyénot, Naville and Ponse (1925) described metacercariae which they found in a single, formalin-preserved specimen of *C. maenas* taken at Boulogne-sur-Mer, France. The cysts were oval, 0.40 by 0.35 mm., and the larvae were identified as *Spelotrema carcini* Lebour. These authors accepted Lebour's (1912) account, designating *C. ubiquita* as the larva of *S. excellens,* but noted that when computed from the stated magnification of the figure, the metacercaria portrayed by M'Intosh was only 0.13 by 0.16 mm. As noted earlier, M'Intosh gave no measurements and the presumed error in magnification may be explained by reduction in size of the drawing on publication. The French authors also described larger cysts, 0.90–1.2 mm. in diameter, in which the walls were weakened and the trematode larvae were filled with spores of a microsporidian, *Nosema* (*Plistophora*) *spelotremae* n. sp.

Stunkard (1932) described cercariae from *Littorina saxatilis* and *L. littorea* at Roscoff, France as *Cercaria ubiquitoides.* Although the larvae were very similar to *C. ubiquita,* slight differences between these specimens and Miss Lebour's description prevented their allocation to that species. In *C. ubiquita* Lebour described two ducts on each side, "which run up the body springing from two masses of large cells which occupy the greater part of the body." In her figure, Lebour showed six gland cells on each side with the two median ducts crossing to open on opposite sides of the body. The cercariae at Roscoff were described as having four penetration glands on each side of the body, with ducts which lead forward, three associated in a common bundle that passes along the lateral face of the oral sucker while the other duct lies more mediad and passes over the sucker. The penetration glands did not stain with neutral red but the secretory granules were clearly visible. These granules, colorless in the cell bodies, stained a deep blood-red in the terminal portions of the ducts and frequently accumulated there to form enlargements. Stunkard also described metacercariae from *C. maenas* and *Porcellana longicornis,* which were referred provisionally to the genus *Spelotrema,* but no attempt was made to relate them to *C. ubiquitoides.*

Rees (1936) described an ubiquitous cercaria from *L. rudis, L. obtusata* and *L. littorea* collected at Aberystwyth in February and March, 1935. He noted that the larvae were almost identical with those described by Stunkard (1932) from *L. rudis* and *L. littorea* at Roscoff. There were slight differences in measurement and other differences concerned the penetration glands, which in the specimens studied by Rees were, "more distinct, not lobed and the most anterior pair of cells is separated from the other three pairs." Rees declared (p. 621), "It is difficult to determine whether these differences are individual or specific because there are so few larval characters in cercariae of the Ubiquita group which can be

used for separating species. The problem is rendered more difficult by the close resemblance of the adult trematodes (species of the genus *Spelotrema*)." In general, the measurements given by Rees agree with or overlap those given for *C. ubiquitoides,* except for the length of the stylet which measured 28 microns against "about 25 microns" in *C. ubiquitoides.* The size, shape and relative position of the gland cells are altered by degree of maturity of the larva, by contractions of the body musculature, and by the emission of secretion into their ducts. Since the ducts from the most anterior pair of penetration glands are separate from the ducts of the other cells, the cell bodies may be somewhat removed. The observation by Rees that only the two anterior pairs of cells and ducts take up neutral red stain, whereas the others do not, is a significant contribution to knowledge of the species. Obviously, I failed to note this feature in *C. ubiquitoides,* but the staining reaction differs with the constitution, concentration, age and condition of the neutral red solution. Accordingly, I am disposed to regard the larvae described by Rees and *C. ubiquitoides* as specifically identical. Since each penetration gland has its own duct, the account of Lebour is not precise and the lateral one of the two reported ducts on each side of the body is almost certainly a bundle of three ducts. Furthermore, it appears that *C. ubiquitoides* can not be distinguished from *C. ubiquita* Lebour, 1907, and the name should be suppressed as a synonym. Lewis (1926) had reported *Spelotrema simile* as very common in gulls of the Aberystwyth area and the finding was confirmed by Rees (1936) who stated (p. 624), "The unusually high percentage infestation of *Littorina rudis* with this cercaria, together with the high percentage of gulls parasitized by *Spelotrema simile,* suggests that this may be the larval form of this species."

Cable and Hunninen (1938) described the successive stages in the life-cycle of a trematode which they identified as a new species, *Spelotrema nicolli.* The asexual generations were in the snail, *Bittium alternatum,* the metacercariae in the blue crab, *Callinectes sapidus,* and the sexual generation was developed in young herring gulls, *Larus argentatus.* These authors (1940) gave a more complete description of *S. nicolli,* the adult of which was compared with that of *S. pygmaeum, S. claviforme, S. simile, S. excellens* and *S. brevicaeca.* In size and size of organs, *S. nicolli* agrees closely with *S. simile;* the major difference is in the size of the male papilla, which is much smaller and similar to that of *S. pygmaeum.* The metacercariae were found only in certain slender fibers which extend from the viscera to the bases of the legs. Cysts increase from 0.05 to 0.50 mm. in diameter; the metacercariae become almost as large as the adults; the excretory formula is 2 [(2 + 2) + (2 + 2)], which persists in the adult stage. The cercaria agrees closely with the description of *C. ubiquitoides* and of the species described by Rees; the major difference is the shorter length of the stylet. *Spelotrema nicolli* is identified by the size of the male papilla, the location of the metacercariae, and the taxonomic position of the first and second intermediate hosts.

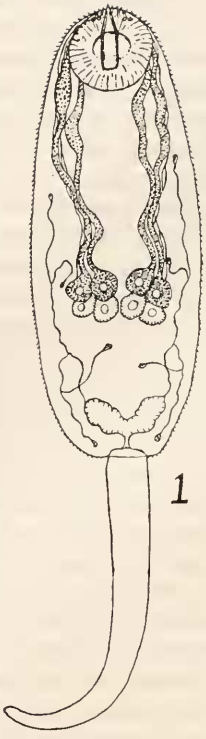
Timon-David (1949) reported metacercariae from the hepatopancreas of *C. maenas* in the Mediterranean at Marseille. The cysts were oval and on this feature and their size, they were identified as *Spelotrema carcini* Lebour.

The present report covers part of a project on clam investigation conducted by the U. S. Fish and Wildlife Service. Since the green or shore-crab, *C. maenas,* is a serious predator of *Mya arenaria* on the New England coast, a survey of its parasites was undertaken in an attempt to determine whether or not some of them

might serve as possible means of biological control. It has long been known that *C. maenas* in the Woods Hole area is infected by an undetermined, encysted metacercaria and experiments have been conducted to discover its identity, life-history, and biology. Metacercariae were fed to white mice and excysted specimens (Fig. 6) recovered after 24 hours. Other cysts were fed to recently hatched, uninfected birds, *Sterna hirundo* and *Larus argentatus*. Large numbers of worms were recovered, including all stages from juvenile to fully mature specimens. The structure of the metacercariae, especially of the smaller and recently excysted ones, suggested that they may be specifically identical with a minute, stylet-bearing cercaria (Figs. 1, 1a) reported by Stunkard (1950), which occurs in *Littorina obtusata, L. saxatilis,* and rarely in *L. littorea*. Small green crabs were exposed to these cercariae and became heavily infected; enormous numbers of worms entered their tissues and developed to metacercariae, identical with those of natural infections. Small crabs exposed continuously with six to eight infected snails died in 10 to 20 days, and on dissection, each one yielded thousands of larvae. The parasites were present throughout the body of the crab, but the heaviest concentration was in the digestive gland. When first encysted, the cyst is oval, the wall is very thin, and the stylet persists for a long time. Eggs of the parasite, recovered from the droppings of terns and gulls, and others teased from the uteri of gravid worms, were used to infect specimens of *L. obtusata,* collected near the laboratory from an area which is not frequented by birds and where examination of 200 snails showed no infection. The eggs were embryonated under running sea water for seven days and then spread on fronds of *Fucus* which were allowed to partially dry to ensure that the eggs would become attached to the slimy surface of the alga. These fronds were placed with 10 small specimens of *L. obtusata* in a gallon jar with a small opening and sharply curved upper shoulders, half filled with sea water and provided with a stream of fine bubbles from an air pump. The water was not changed for ten days and subsequently on alternate days the top water was siphoned off and replaced with fresh sea water. Since the eggs of microphallid trematodes do not hatch until they are eaten by a suitable snail, and since from the design of the experiment it is impossible to tell when eggs were eaten, the age of an infection is not known precisely. The snails were first exposed on July 31, and on October 1, five snails that were alive in the jar were crushed and examined. Three of them were infected; two with large numbers of small daughter sporocysts but not yet producing cercariae, and the other contained three primary or mother sporocysts (Fig. 2) but no free daughters. The early stages of the infections afford clear evidence of their experimental nature and the complete life-cycle was thus consummated by laboratory infection of both intermediate and final hosts.

The adult worms agree so completely with the descriptions of *Microphallus similis* (syn. *Spelotrema simile*) as given by Jägerskiöld (1900) and Odhner (1905), that they are assigned to that species. The experimental demonstration of the life-cycle confirms the suggestion of Rees (1936), that *Cercaria ubiquita* Lebour is the cercarial stage of *M. similis*. Actually, the adults were described by Jägerskiöld (1900), the metacercariae by M'Intosh (1865), and the cercariae by Lebour (1907). Whether the specimens described by Nicoll (1907) as *S. excellens* and *S. feriatum* are specifically distinct remains to be determined.

Attempts to infect *Limulus polyphemus* were unsuccessful. Three small horse-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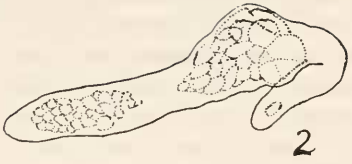
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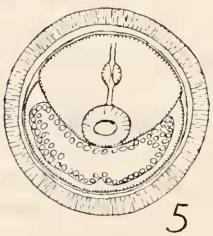
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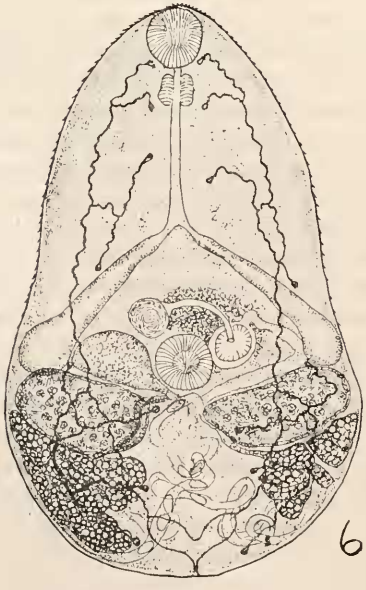
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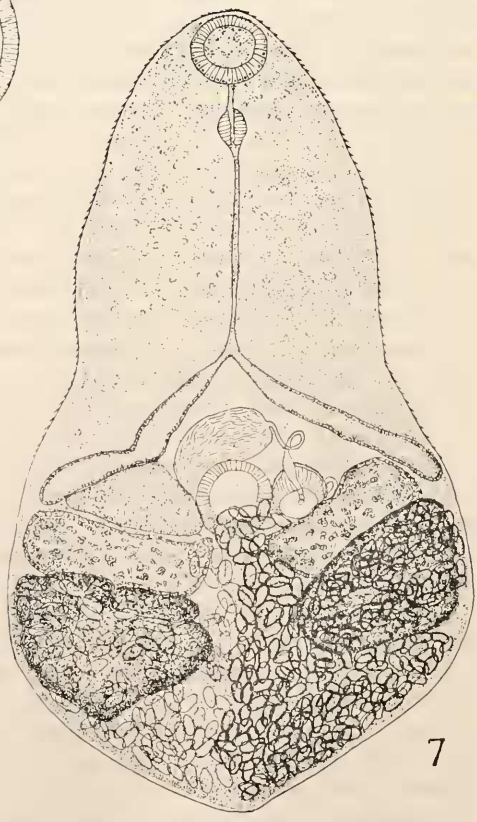
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shoe crabs with carapace widths of 17, 19, and 21 mm., collected near Orleans, Massachusetts, were exposed to cercariae for two weeks. On dissection, there were no recently entered, unencysted larvae although all three harbored mature cysts of *Microphallus limuli* in their livers. This finding supplements that of Stunkard (1953), who reported full-sized cysts in small *L. polyphemus*. In paragraph 3, line 4, of that report, 3 mm. should read 30 mm.

Description of Stages in the Life-Cycle

Measurements in millimeters

Adults (Fig. 7)

Egg-bearing specimens from gulls and terns are about the same size. Fixed, stained and mounted they measured: length, 0.36–0.7; width, 0.22–0.36; acetabulum, 0.048–0.065; oral sucker, 0.05–0.065; pharynx, 0.02–0.03; male papilla, diameter 0.038–0.058; testes, 0.10 × 0.06 to 0.16 × 0.09; seminal vesicle, 0.06 × 0.04 to 0.09 × 0.064; ovary, 0.08 × 0.05 to 0.1 × 0.062; eggs colorless on the ovarian side, yellow on the antovarian side, 0.022–0.027 × 0.011–0.012, often collapsed and somewhat distorted in fixed and stained specimens.

The body is oval to pyriform and either end may be wider; often there is a slight constriction between the more mobile forebody and the inert hindbody which is filled with reproductive organs and eggs. The dermomuscular wall is thin and weakly developed; it consists of the usual circular, longitudinal and oblique layers, but the fibers are delicate and relatively few. The edges of the forebody tend to turn ventrad, forming an adhesive cup. The cuticula bears flattened spines which are conspicuous on the anterior half of the body but become smaller and sparser posteriorly. The suckers are approximately equal in size; either may appear larger, depending on the degree of contraction by the sphincter and the size of the orifice. The esophagus is long and when the body is extended, it may be much longer than the ceca. The ceca are almost straight; they diverge at an obtuse angle and terminate at the acetabular level. The anterior tips are constricted and lined with cuticula, continuous with that of the esophagus. The excretory system is unchanged from the metacercarial stage and has the formula $2[\ (2+2)\ +\ (2+2)\ ]$. The testes are dorsal, lateral, opposite; the vasa deferentia arise at the medial, anterior faces, pass mediad and anteriad where they unite to form the seminal

Figure 1. Cercaria from *L. obtusata,* free-hand drawing of specimen stained with Nile blue sulphate; 1a, lateral aspect of stylet.

Figure 2. Primary sporocyst from *L. obtusata,* experimental infection; fixed and stained specimen, length 0.30 mm.

Figure 3. Daughter or secondary sporocyst from *L. obtusata;* natural infection; fixed and stained specimen, length, 0.375 mm.

Figure 4. Cross-section of a mature worm to show the relative position and size of the acetabulum and of the male papilla. The metraterm opens into the genital atrium near the base of the papilla and in front of the left testis whose anterior portion appears in the section; width of the worm, 0.28 mm.

Figure 5. Metacercaria from *C. maenas,* natural infection; cyst 0.46 mm. in diameter.

Figure 6. Specimen, much flattened to study the excretory system, from intestine of a white mouse, 24 hours after the cyst was eaten; length 0.55 mm.

Figure 7. Adult specimen from intestine of *Sterna hirundo,* experimental infection; one of the largest specimens, flattened under a cover glass, fixed and stained, length 0.69 mm.

vesicle, an oval sac which lies anterior and dorsal to the acetabulum. A coiled ejaculatory duct, enclosed in glandular cells, leads from the vesicle to the muscular male papilla which almost fills the genital atrium, situated at the left of the acetabulum. The ovary is oval to triangular, located on the right side of the body, between the seminal vesicle and the testis and cecum of the right side. The oviduct arises from the median posterior region, coils posteriad and ventrad where it expands to form a fertilization space from which Laurer's canal winds to the dorsal surface of the body, opening in the midline just behind the acetabulum. The oviduct then turns dorsad and anteriad, receives a short common vitelline duct and enlarges to form the ootype, lined with cilia and enclosed in the cells of Mehlis' gland. The vitellaria are large, lobed glands, situated below and behind the testes; ducts from the two sides pass mediad, anteriad and dorsad, uniting to form the common vitelline duct which discharges into the initial portion of the ootype. The uterus passes backward from the ootype almost to the posterior end of the body and then loops forward in coils on the ovarian side of the body as far as the end of the digestive cecum, then backward almost to the posterior end of the body where it crosses to the opposite side and forms a corresponding series of loops on the left side, with the terminal metratermal portion emptying into the left side of the genital atrium (Fig. 4). The extent of the uterus anteriorly is determined by the number of eggs; in certain specimens the uterine coils may be below and behind the testes whereas in others the coils may underlie the ends of the digestive ceca.

The egg and miracidium

Egg-production begins almost immediately after the metacercaria is eaten by the final host. Figure 6 shows a worm which, fed as a metacercaria to a white mouse twenty-four hours earlier, already has eggs in the initial portion of the uterus. At first the egg-shell is thin, flexible and transparent; but as the eggs traverse the uterus, the shells become thicker, harder, and bright yellow. This coloration of the shell obscures the larva in living eggs, but development can be followed by study of serial sections of gravid worms. The egg is operculate and the ovum is situated toward the opercular end of the egg. In eggs near the metraterm, the miracidium appears to be fully formed, but eggs used for infection experiments were kept for a week in running sea water to insure fully mature larvae. The miracidia emerge only after the eggs have been ingested by the snail host.

Sporocyst generations (Figs. 2, 3)

The amount of experimental material is limited, but three primary sporocysts were removed from a specimen of *L. obtusata* two months after exposure to eggs of *M. similis*. These sporocysts were sluggish, oval to cylindrical and 0.25 to 0.40 mm. long. One of them, fixed and stained, is shown in Figure 2. The presence in them of recognizable daughters identified them as sporocysts of the mother or primary generation. Daughter sporocysts obtained from two experimental infections were young, small, and very numerous. Daughter sporocysts of natural infection measured 0.10 to 0.60 mm. in length; they are oval, occur in

large numbers, more than one hundred in a single snail. The wall often contains a yellowish pigment; it is thin and in older sporocysts change of shape results from movement of contained cercariae. Figure 3 was made from a daughter sporocyst, 0.375 mm. long.

Cercaria (Figs. 1, 1a)

Body length, 0.1–0.22 mm.; width, 0.02–0.05 mm.; the larva is very thin, delicate, colorless. The tail is 0.01–0.012 mm. wide at the base; contracted it is 0.05 mm. long, with fine cuticular annulations; extended it may be 0.25 mm. long and very slender. There is no acetabulum; the larvae move by strokes of the tail but are unable to creep. They swim upward and sink when motionless. In swimming the body is contracted, bent ventrad, while the tail is extended and lashes violently. The body is covered with cuticular spines and the dorsal wall of the sucker bears a stylet, 0.023–0.026 mm. long and 0.009 mm. wide. The stylet (Fig. 1a) is asymmetrical in lateral aspect. The oral sucker measures 0.025–0.032 mm.; other parts of the digestive system are not yet developed. The body contains numerous cystogenous glands and on either side, near the middle, there are four penetration glands. The two anterior cells on each side differ from the posterior ones; the difference is demonstrated by the use of neutral red or Nile blue sulphate solutions, especially the latter, which stain the secretory granules of the anterior cells selectively while the contents of the posterior cells do not take the stain. Ducts from the anterior pair of cells pass forward beside those from the other cells for a short distance, but about halfway to the mouth they separate from the others and pass more mediad, crossing the dorsal side of the oral sucker, while the ducts from the other three penetration gland cells form a bundle that continues forward and passes at the side of the sucker. The ducts from the two anterior pairs of cells open to the surface ventrally, below the tip of the stylet, whereas the ducts from the posterior pairs open more anteriorly and at the sides of the tip of the stylet. The excretory system is shown in Figure 1; the vesicle is U- or V-shaped and the flame-cell formula is $2[\,(1+1)+(1+1)\,]$. The cercariae are carried by respiratory currents into the gill chambers of *C. maenas* where they enter the body at the bases of the gills and possibly at other non-cuticularized places, pass by way of the vascular system to all parts of the body, and localize principally in the connective tissue of the digestive gland.

Metacercaria (Fig. 5)

The cysts increase in size and measure from 0.05 to 0.55 mm. in diameter; when the cercariae encyst they are bent ventrally; the cyst is oval and the wall is very thin and flexible. The stylet is retained for some time and readily identifies the species. As the metacercaria grows, the digestive tract and actabulum are formed and the number of flame-cells is doubled. As the worm grows, the cyst becomes spherical and the wall increases in thickness. In a full grown cyst, the cavity may be 0.35–0.40 mm. in diameter and the wall consists of two layers, an inner hyaline one which may be resolved into strata and attains a thickness of 0.02 mm., and an outer radially striated layer which increases to a thickness of 0.06 mm. The substance of this layer, after digestion in a pancreatin solution,

appears to consist of parallel prisms. The worms become almost full grown as metacercariae; the adults increase in size only by the further activity of the reproductive organs and the accumulation of eggs in the uterus. When younger and smaller metacercariae are eaten, the worms become gravid at a smaller size than when the metacercariae are older. The excretory vesicle of older metacercariae contains spherical concretions, the excretory wastes accumulated during the period of encystment.

Summary

1. The life-history of *Microphallus similis* has been worked out by experimental infection of both intermediate and final hosts.

2. Encysted metacercariae from *Carcinides maenas* developed to sexual maturity in *Larus argentatus* and *Sterna hirundo*. Eggs of the parasite developed in these hosts were used to infect *Littorina obtusata*. Two generations of sporocysts were recovered.

3. *Littorina saxatilis* and *Littorina littorea* also harbor the asexual generations at Woods Hole, Massachusetts.

4. The cercariae are minute, stylet-bearing monostomes and small green crabs, *C. maenas*, exposed to these cercariae became heavily infected; enormous numbers of larvae entered the tissues and developed into metacercariae identical with those of natural infections. Small crabs, each exposed continuously to the cercariae from six to eight infected snails, died in ten to twenty days and on dissection each yielded thousands of larvae.

5. The stages in the life-cycle of the parasite agree with descriptions by European investigators of corresponding stages: the metacercariae with metacercariae from *C. maenas*, described but not named by M'Intosh (1865); the adults with *M. similis* from Swedish gulls, described and named by Jägerskiöld (1900); and the cercariae with *Cercaria ubiquita* Lebour, 1907. The identity of these parasites as stages in the life-cycle of a single species is predicated.

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